Influence of Potassium on Carotenoid Content of Tomato Fruit[1,2]

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Abstract. Tomato plants were grown in sand culture at 8 levels of K ranging from 0 to 10 meq per liter of nutrient solution. Fully ripened fruit were picked and rated internally and externally for abnormal pigmentation. Total carotenoids were analyzed and the extract fractionated into 3 groups: hydrocarbon, monohydroxy and polyhydroxy. The hydrocarbon fraction was further separated chromatographically into 6 individual pigments. Most of the carotenoids, lycopene in particular, generally increased with increasing K concn. A notable exception to this pattern was β-carotene which decreased with increasing K concn. Possible modes of action of K are discussed.

The development of red color in tomato fruit during ripening mainly due to the synthesis of carotenoid pigments, articularly lycopene. The lack of uniform coloration is a ommon ripening disorder often referred to as "blotchy pening". The symptomatology of blotchy ripening was studied y Sadik and Minges (25), who found that "white tissue" in the ericarp is the basic abnormality associated with non-uniform igmentation of the fruit.

Imperfect ripening of the fruit has been attributed to light ffects (2), water relations (26), and nutritional disorders. articularly K deficiency[4] (3, 12). In the first paper of this eries, Ozbun et al. (19) speculated that K might be involved in ie synthesis of lycopene. However, Denisen (6) had previously eported that K nutrition did not influence the lycopene content f tomato fruit. Literature on the effect of K on carotenoid ynthesis is not conclusive. Potassium deficiency was found to ncrease (11), decrease (16) or have no effect (15) on the arotenoid content of various leaf materials.

Our objective was to determine the effect of K nutrition on ie carotenoid content of tomato fruit and to establish the elationship between pigment level and the expression of blotchy ripening".

Materials and Methods

Plant material. Tomato seeds (*Lycopersicon esculentum* cv. Fireball) were germinated in sand and the resulting seedlings vatered daily with Hoagland no. 1 nutrient solution (10). Upon eaching a height of 20-25 cm, the plants were transferred to 30 m diam plastic pots filled with Quartzite-Silica sand, grade no. , and thereafter grown under glass. Greenhouse temp was 18°C ight and 24-27° day. The plants were staked and pruned, llowing a single shoot to develop with 4 flower clusters. Before reatment initiation, the plants were watered daily with 1 liter f Hoagland no. 1 solution except on the 3rd and 7th day of the veek when they received tap water. Treatments were started vhen first fruit were 1.0-1.5 cm in diam. The 8 treatment olutions were modified Hoagland no. 1 solutions in which the K concentration (concn) was varied from 0 to 10 meq of K per ter. Total salt concn was kept constant by varying the level of NaCl. Fruit were allowed to ripen on the plant.

Fruit rating. After harvest, red fruit were rated for ripening disorders. We used the numerical rating system for fruit discoloration as proposed by Minges and Sadik (18). All external symptoms of blotchy ripening were considered when assigning the fruit an external rating. White tissue was the internal symptom rated. To obtain this rating the fruit were cut in cross-section midway between the stem and blossom end. In both internal and external grading, a rating of 5 indicates a fruit free of any disorder and a rating of 1 a severely affected fruit.

All fruit from each treatment (6 plants/treatment with 8-10 fruit/plant) were homogenized in a large blender. Twenty-five g aliquots of the homogenate were frozen at -10° for further analysis.

Pigment extraction. Frozen fruit samples of about 25 g were thawed in an air-tight container, weighed and homogenized in a Virtis high-speed homogenizer with an equal amount of acetone. The homogenate was filtered through filter paper coated with filter-aid (Celite 545) on a Buchner funnel. The same acetone treatment was repeated to remove all water from the tissue. The pulp was then extracted with several portions of acetone-hexane (50:50) to yield the carotenoids. Extraction was repeated until the pulp and the last filtrate were clear. The extracting solutions were combined in a separatory funnel and washed several times with water to remove the acetone. The hexane solution was then saponified by shaking several minutes with ¼ vol of saturated KOH in methanol. The unsaponifiable fraction containing the carotenoids was washed free of alcohol and alkali with distilled water and dried by filtering through an 18 x 100 mm column of anhydrous sodium sulfate. An aliquot was then removed for spectral analysis to determine the total carotenoid content.

Chromatography. The extract was fractionated on a silica gel-methanol column according to Purcell (21) and 3 fractions obtained; carotene hydrocarbons, monohydroxy and polyhydroxy carotenoids. Each fraction was evaporated to dryness under vacuum and brought to vol with hexane in case of hydrocarbon and monohydroxy carotenoid fractions and with methanol for polyhydroxy carotenoids.

The hydrocarbon carotenoid solution was chromatographed on a tightly packed 18 x 200 mm column of MgO: Celite 545 (1:1, w:w), with slight pressure applied at the top. The column was developed by increasing the concn of acetone in hexane stepwise up to 10%. Phytoene, phytofluene, β-carotene, and ξ-carotene were respectively eluted and collected as separate fractions. The γ-carotene fraction was then eluted by adding 1% methanol to the 10% acetone-hexane solvent. The elution of lycopene was with 2% methanol in 10% acetone-hexane. Once the main band of lycopene was eluted, the column was further washed with large amounts of solvent to minimize previously reported losses of this pigment (17). Upon completion of the chromatography, individual fractions were evaporated to dryness and made up to known vol in hexane.

[1]Received April 5, 1971. Paper No. 608 - Department of Vegetable Crops, Cornell University, Ithaca, New York.

[2]One of us (M. J. Trudel) held a Quebec Agricultural Research Council Fellowship during his participation in this work. The excellent technical assistance of Miss S. Altschuller is gratefully acknowledged.

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[4]Boutonnet, C. E. 1966. The influence of certain nutritional treatments on blotchy ripening of tomatoes in the field and greenhouse. M.S. thesis. Cornell University, Ithaca, N. Y.

Spectrophotometric determinations. Spectra of the carotenoids in hexane were obtained with a Perkin-Elmer 202 recording spectrophotometer. The individual pigment concn was determined using known absorption coefficients specific to each of these pigments (4, 17). The absorbance at maximum absorption was measured and a specific absorption coefficient of 250 mg^{-1} cm^{-1} used (22) for calculating the amount of pigment in the mixtures (total carotenoids and the fractions eluted from the silica gel-methanol column).

Potassium analysis. Dry samples were ashed in a muffle oven at 450° for 12 hr. The residue was digested with 1.2 N HCl with low heating until the solution cleared. The solutions were analyzed on a Perkin-Elmer atomic absorption spectrophotometer according to the method of Berry and Johnson (1).

Results and Discussion

The K content of both fruit and petiole increased with increasing concn of K in the nutrient solution (Table 1). The ratio of the K content of the fruit to that of the petiole generally decreased with increasing concn of K in the solution. This suggests that the fruit is quite efficient in monopolyzing K under conditions of severe deficiency.

Table 1. The K content of tomato fruit and petioles as influenced by K concn of the nutrient solution.

Meq K/l nutrient solution	Meq K per g dry wt		Fruit/petiole
	Petiole	Fruit	
0	0.105	0.347	3.30
0.5	0.150	0.428	2.85
1	0.150	0.362	2.41
2	0.265	0.510	1.92
4	0.280	0.677	2.41
6	0.500	0.741	1.48
8	0.610	0.852	1.40
10	0.927	1.071	1.15

The fruit were rated internally and externally for ripening disorders. The numerical rating system for "white tissue" proposed by Minges and Sadik (18) was found to be quite satisfactory with the values being closely correlated to the pigment concn of the fruit (Table 2). A linear correlation was found between total carotenoids and rating values. Both correlations are significant at the 1% level.

Table 2. Rating for ripening disorder (discoloration) of tomato fruit at 8 levels of K as related to the carotenoid content[a].

Meq K/l nutrient solution	Total carotenoids μg/g fr. wt	Fruit rating[b]	
		Internal	External
0	71.58 ± 8.10	1.86	3.31
0.5	64.97 ± 1.80	1.45	2.95
1	74.86 ± 2.47	2.19	3.81
2	91.48 ± 3.47	2.41	3.65
4	91.92 ± 1.65	2.44	3.98
6	110.50 ± 1.61	2.51	4.07
8	111.50 ± 9.74	2.86	4.21
10	104.50 ± 7.13	3.36	3.98

[a]Fruit rating correlations with total carotenoids are significant at the 1% level (internal, r = 0.852; external, r = 0.878).
[b]Each value is a mean for fruit from 6 plants. See "Materials and Methods" for description of rating system.

Total carotenoids generally increase with increasing amounts of K in the nutrient solution. A maximum (max) is reached at 8 meq with a slight decrease occurring at 10 meq of K. The differences in amount of pigment for the fruit grown at the 3 lower concn of K were not significant.

Fractionation of total carotenoids into 3 classes of pigments showed that hydrocarbon carotenoids varied with the K level essentially as the total carotenoids (Table 3). The amounts of monohydroxy and polyhydroxy carotenoids were small and the standard error was rather large. Levels of these 2 classes of pigments were not influenced by the treatments.

Table 3. Carotenoid pigments of tomato fruit at 8 levels of K.

Meq K/l nutrient solution	μg/g fr. wt[a]		
	Hydrocarbon carotenoids	Monohydroxy carotenoids	Polyhydroxy carotenoids
0	62.44 ± 2.09	2.91 ± 0.42	1.07 ± 0.08
0.5	55.52 ± 1.67	2.55 ± 0.21	1.56 ± 0.14
1	66.16 ± 1.59	2.78 ± 0.43	1.10 ± 0.14
2	77.04 ± 2.33	3.30 ± 0.49	1.25 ± 0.27
4	77.66 ± 3.99	2.98 ± 0.29	1.14 ± 0.13
6	89.80 ± 1.89	3.35 ± 0.34	1.24 ± 0.21
8	94.43 ± 1.60	2.65 ± 0.25	1.20 ± 0.29
10	81.80 ± 1.44	2.74 ± 0.18	1.11 ± 0.19

[a]Means of 4 analyses and standard error.

The hydrocarbon carotenoid fraction contributes most of the color to the ripe fruit. Consequently, it was further analyzed by separation into 2 colorless polyenes (phytoene and phytofluene), 3 carotenes (β, ξ, and γ) and finally lycopene. Phytoene, phytofluene, and ξ-carotene follow a similar trend with increasing K (Table 4). For any of these pigments, 2 meq of K per liter of nutrient solution seems to be the critical level beyond which changes in K had no significance. In most instances, the amounts of these 3 pigments in fruit from plants grown at the 0.5 meq level seemed to be abnormally low. It should be noted, however, that fruit from this treatment were assigned a lower rating both internally and externally (Table 2). High concn of K influenced γ-carotene since a decline was observed following a max at 6 meq level. It should be pointed out that this particular fraction seemed to be contaminated with some other pigments which were in too minute amount to be effectively separated by the technique used. These 2 minor pigments were later found to be neurosporene and δ-carotene.

Lycopene content rises sharply as the K level in the nutrient solution is increased with a max at 8 meq and a slight decrease at the 10 meq level (Table 4). However, β-carotene follows an inverse pattern and rapidly decreases at K concn higher than 1 meq per liter of nutrient solution (Table 4). The general trend of these 2 pigments are evidently opposite. The reports on the effect of K nutrition on carotene content of various plant material were found to be inconsistent throughout the literature. Our data with 'Fireball' tomato fruit shows that a deficiency in K increases the concn of β-carotene in the fruit and decreases the concn of other carotenoids, particularly lycopene. The reported data are representative of a number of similar experiments showing the same trends.

It is clear that lycopene is the pigment which is most sensitive to low levels of K. The mechanism whereby K interferes with lycopene synthesis appears to be complex and not readily understood. Since K is an essential co-factor in protein synthesis (28), one can assume that under conditions of severe deficiency, the enzyme level is lowered. This could lead to reduced rates of enzymatic reactions involved in carotenoid and precursor synthesis.

A change in protein synthesis could also lead to abnormal plastid membranes. Structural changes in chloroplasts of K-deficient plants were reported by Thompson and Weier (27). If membranes of the fruit plastids were affected in a similar way, the permeability properties of these membranes could be altered. As a consequence, changes in the rate of precursor flow across the plastid membranes could influence the rate of carotenoid synthesis inside the chromoplasts.

Furthermore, acetic thiokinase, the enzyme responsible for the formation of acetyl CoA, has been shown to require K for activity (9). Since the condensation of two molecules of acetyl CoA is the first step in the classical pathway of carotenoid precursor formation (20), a lowering of the carotenoid level

Table 4. Hydrocarbon carotenoid content of fruit from tomato plants grown at 8 levels of K.

Meq K/l nutrient solution	μg/g fr. wt[a]					
	Phytoene	Phytofluene	β-carotene	ξ-carotene	γ-carotene	Lycopene
0	11.76 ±0.13	4.09 ± 0.08	3.48 ± 0.03	0.76 ± 0.08	1.38 ± 0.05	36.81 ± 1.23
0.5	9.66 ±0.44	3.03 ± 0.09	3.36 ± 0.03	0.63 ± 0.01	1.18 ± 0.08	33.75 ± 1.34
1	12.70 ±0.57	4.14 ± 0.11	3.62 ± 0.05	0.91 ± 0.06	1.44 ± 0.08	41.88 ± 1.57
2	16.25 ±0.54	5.45 ± 0.12	3.07 ± 0.01	1.08 ± 0.01	1.51 ± 0.09	53.60 ± 5.27
4	15.25 ±0.17	4.92 ± 0.05	2.80 ± 0.06	0.97 ± 0.05	1.65 ± 0.08	52.67 ± 0.88
6	14.73 ±1.66	4.99 ± 0.03	2.80 ± 0.07	1.04 ± 0.01	1.76 ± 0.07	59.33 ± 1.63
8	15.14 ±0.63	4.80 ± 0.03	2.56 ± 0.01	0.92 ± 0.01	1.51 ± 0.09	61.51 ± 1.46
10	16.35 ±1.49	5.32 ± 0.16	2.36 ± 0.08	0.98 ± 0.06	1.47 ± 0.14	52.39 ± 3.63

[a]Means of 4 analyses and standard error.

ould be expected under low K. Any of the above cited factors r a combination could account for the general reduction bserved in the amount of total carotenoids of K-deficient fruit.

The scheme of carotenoid synthesis most often referred to iggests that lycopene and β-carotene are synthesized on ifferent branches of the pathway (7, 13). If the scheme is valid, ur data suggests that with severe K deficiency, more of the lready lowered intermediates are channelled through the -carotene branch at the expense of lycopene synthesis. If so, ne would expect γ-carotene to follow a pattern somewhat milar to β-carotene since it is assumed to be an intermediate in s formation. Our data do not support this as γ-carotene llows a pattern similar to that for lycopene except for a faster op at higher levels of K. However, the contamination of -carotene with minor pigments forbids definite rejection of the ypothesis.

Evidence has been presented for the conversion of C^{14}-carotene to lycopene in carrot plastids (5) and the reverse action in tomato plastids (14). If lycopene can be synthesized om β-carotene in tomato fruit, our data could suggest that the nzyme responsible for this conversion may be K activated. hat is, under normal K nutrition, β-carotene is converted to copene while under conditions of severe deficiency, the action is stopped or drastically reduced, the net result being accumulation of β-carotene.

The effect of K deficiency on carotenoid synthesis seems to different from high temp (8) and DMSO (23, 24) inhibition lycopene synthesis. High temp and DMSO inhibit the nthesis of lycopene while the level of β-carotene remains changed. This suggests a selective inhibition of isoenzymes om parallel pathways leading to the formation of these 2 gments (23). However, in K deficiency, the inhibition of copene synthesis is concomitant to the accumulation of arotene as a result of either the activation of its synthesis or e inhibition of its metabolic transformations as suggested ove.

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